EXHIBIT 8

HUMAN GENE TRANSFER/THERAPY PROTOCOL

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Gene Therapy of Cancer: A Pilot Study of IL-4-Gene-Modified Antitumor Vaccines

Principal Investigators:

Michael T. Lotze, M.D.
Joshua T. Rubin, M.D.

Associate Investigators:

Oncologic Surgery
Sally Carty, M.D., Ph.D.
Howard Edington, M.D.
Peter Ferson, M.D.
Rodney Landreneau, M.D.
Barbara Pippin, B.A.
Mitchell Posner, M.D.
Deborah Rosenfelder, R.N.
Charles Watson, M.D.
Norman Wolmark, M.D.

Medical Oncology
Timothy Carlos, M.D.
John Kirkwood, M.D.
Barry Lembersky, M.D.
Theodore Logan, M.D.

IMDL/CT
Theresa Whiteside, Ph.D.
Elaine Elder, M.S.

Genetic Therapy, Incorporated
Robert C. Moen, M.D., Ph.D.
William Jacob, Ph.D.

Ethics
Rosa Lynn Pinkus, Ph.D.

Statistics
John Bryant, Ph.D.

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1.0 INTRODUCTION AND BACKGROUND

Local therapies, such as surgery and radiation, rarely cure patients with disseminated cancer. Lamentably, our ability to alter the natural history of metastatic cancer using available chemotherapeutics is also limited and it is apparent that additional therapeutic strategies are needed. We, and others, have previously demonstrated that interleukin-2 (IL-2)-based immunotherapy induces clinically significant tumor regression in 20 to 30 percent of treated patients who have metastatic melanoma or renal cell carcinoma, respectively (1). Responses have been noted, albeit much less frequently, among treated patients who have other malignancies including colon carcinoma, ovarian carcinoma, breast carcinoma and non-Hodgkin's lymphoma (2). The cost to the individual patient is significant toxicity. Although a small proportion of patients manifest durable clinical responses, prolonged survival, cure, or even palliation have yet to be clearly demonstrated.

An alternative strategy for immunotherapy is suggested by *in vitro* and preclinical murine data showing that the cytokine microenvironment within tumors may determine the outcome of the immune response. In particular, the immunomodulatory cytokine interleukin-4 (IL-4) has a central role in the immune response to tumors in certain murine models. Implantation of the murine mastocytoma P815 into the anterior chamber of the eye results in progressive growth of this tumor while implantation into the subconjunctival sac of the eye results in tumor rejection. Cytolytic precursor cells residing in both sites secrete IL-2. However, only those isolated from the rejecting site (subconjunctival sac) elaborate IL-4 upon exposure to tumor (3). Bosco, *et al* (4), have injected IL-4 into the region of tumor-draining lymph nodes of mice bearing either a fibrosarcoma or a mammary adenocarcinoma. As little as 0.1 picogram per day induced tumor regression and protective immunity to subsequent tumor challenge. Tepper *et al* (5) demonstrated that an invariably lethal murine B-cell lymphoma or a mammary adenocarcinoma, both transfected with the IL-4 gene, regressed after a brief period of growth. Golumbek *et al* (6) have observed a similar effect with a spontaneous renal cell carcinoma (Renca) transfected with the gene for IL-4. They extended these observations by demonstrating both protective immunity against tumor rechallenge in mice vaccinated with these gene-modified tumors and tumor regression in a 6 to 9 day tumor model. In contrast to the demonstrated lack of therapeutic efficacy of IL-4 when administered systemically to patients with cancer (unpublished observations), these studies suggest that the constant local production of IL-4 is associated with development of antitumor immunity that may translate to regression of established cancer.

Some of these antitumor immunologic effects have been observed with as little as 0.44 units (U) IL-4/10^6 cells/48 hours (5). However, regression of established tumor has only been associated with a vaccine that elaborated 1500 U/10^5 cells/24 hours (6). In this study, 10^6 cells used for vaccination would have been expected to release 15,000 U of IL-4 daily at the vaccine site. Such high, constant intratumor concentrations of IL-4 can not be attained with systemically administered IL-4 due to the associated toxicity.

The mechanism underlying the antitumor effects of IL-4-transduced tumor vaccines has not been clearly defined. Tumor regression has been associated with a mononuclear infiltrate consisting predominantly of macrophages (5, 6).

Lymphocytes and eosinophils have also been observed (4-6). IL-4 may play a critical role in the homing of these cells to sites of tumor by inducing the expression of the vascular cell adhesion molecule VCAM by the tumor microvascular endothelium (7). Lymphocyte adhesion to these cells is thought to be mediated by interaction through cell surface VLA-4 (7). The effects of IL-4 on macrophages include the upregulation of class II molecule expression (8) and the induction of antigen-presenting capacity (9). T-cell activity is also modulated by IL-4. Specifically, IL-4 increases IL-2 production, increases IL-2 receptor expression, and enhances proliferation of mitogen-activated T cells (10). When IL-4 is combined with IL-2, LAK activity is suppressed while specific antitumor lytic activity is enhanced (11). These findings are concordant with the demonstration by Golumbek et al (6) that antitumor effects are abrogated by depleting mice of CD8+ cells prior to vaccination. Based on these observations, we have targeted tumor vaccines, designed to elaborate IL-4 at the site of vaccination, as a rational approach to immunotherapy.

In light of the limited ability to consistently and quickly generate tumor cell lines for use in these studies, we have identified cultured fibroblasts a cells that can be quickly expanded to large numbers, easily transduced, and used as vehicles to deliver high, constant levels of IL-4 to the vaccine site. We have routinely been able to get 10^6 fibroblasts from small (1cm x 1cm) biopsies of skin after 2 to 3 weeks of culture and to expand them 2 to 4 fold each week thereafter. We plan to take 4cm diameter biopsies that are expected to yield about 10 to 20 fold more cells for these vaccine studies. We have been able to induce fibroblasts growing in log phase to incorporate the gene for IL-4 and produce bioactivity of <0.1 to 10^2 units/10^6 cells/24 hours without selection, and as much as 10^4 U with selection. After irradiation with 5000 rads, these cells slowly involute and disappear after 1-2 weeks of additional culture. Irradiated, autologous fibroblasts transduced with the gene encoding IL-4 will be admixed with irradiated single-cell tumor suspensions and administered intradermally to patients with disseminated cancer.

We already have extensive experience with the preparation of single cell suspensions of tumor for laboratory studies as well as for vaccine trials at the Pittsburgh Cancer Institute. This study is seen as an extension of our previous trials. We also have extensive experience with retroviral transduction of tumor infiltrating lymphocytes (TIL) using the gene that encodes resistance to the neomycin analog, G418. While at the National Cancer Institute, we treated ten patients with gene-marked TIL (12). No toxicities of any kind could be attributed to these cells. The expected toxicities associated with the concomitant administration of IL-2 were seen. Patients received up to 1.45×10^{11} gene-transduced TIL. The percent of cells transduced among the adoptively transferred TIL populations varied between 1 and 11 per cent. In each case, integration and expression of the NeoR gene was demonstrated. Gene-modified TIL could be detected at tumor deposits as long as 64 days after infusion. We have now treated two patients with disseminated cancer at the University of Pittsburgh with IL-2, IL-4, and TIL that have been tranduced with the NeoR gene. Toxicities of treatment were those usually associated with the systemic administration of IL-2 and IL-4 and are primarily those associated with a vascular leak.

2.0 OBJECTIVES

2.1 To determine the period of time over which irradiated, gene-modified fibroblasts elaborate IL-4 in vitro.

2.2 To define the local and systemic toxicity associated with vaccines composed of tumor cells admixed with fibroblasts that have been transduced with the gene encoding IL-4.

2.3 To evaluate the local immune response induced by these vaccines.

2.4 To evaluate the clinical efficacy of these vaccines.

3.0 EXPERIMENTAL DESIGN

3.1 Obtaining Tissue for Use in this Study:

There are a number of obstacles to the timely preparation of tumor vaccines using gene-modified cells from patients with melanoma, breast cancer, renal cell carcinoma, and colon cancer. With the exception of patients with metastatic melanoma, relatively few patients develop accessible subcutaneous metastases. Rather, pulmonary metastatic disease is a far more common site of dissemination. These lesions can now be resected thoracoscopically with little morbidity and a small theoretical risk of mortality (13). This minimally invasive surgical technique makes pulmonary metastasectomy a reasonable approach to obtain tumor tissue for these studies. Some patients who have previously undergone curative resection of their primary malignancy have had their tumor stored in the PCI tissue bank. About 200 of these patients are currently followed in the PCI outpatient facility. In the event that they develop metastatic disease, they would be eligible for this protocol without the need for tumor harvesting.

Subcutaneous metastases and thoracoscopically resected lung metastases will be transported in sterile fashion to the laboratory where they will be processed (appendix 19.1). A small representative piece of tissue will be saved for histopathologic exam. The remaining tissue will be digested as previously described and a single cell suspension made (appendix 19.1). This heterogeneous population of cells will be stored in complete medium containing 90% human AB serum and 10% DMSO at -180°C.

Rapidly proliferating cells are a prerequisite for retroviral gene transduction. Unfortunately, our ability to generate tumor lines from these resected metastases is limited. In order to obtain large numbers of proliferating cells that can be transduced and available for use in a short period of time, we propose to use cultured, autologous fibroblasts grown from skin resected at the time of metastasectomy (19.2). An elipse of skin measuring 4cm by 1cm will be resected from the anterior abdominal wall. This will be performed in sterile fashion in the operating room using either general or local anesthesia depending on the approach to metastasectomy. All wounds will be closed primarily with either sutures or staples. The skin will be transferred in sterile fashion to the laboratory where it will be cleared of fat, divided into 1mm-2mm diameter pieces and plated in T25 flasks containing sterile culture medium and 10% human AB

serum. Cultures will be split 1 to 3 when confluent monolayers develop. After the first passage, the fibroblasts will be transduced and replated as previously described.

3.2 Transduction and Growth of Fibroblasts:

The procedures used here are the same as those used in our previous protocols at the NIH and the PCI, involving the infusion of TIL transduced with the Neo resistance gene (protocol 86-C-183c and 90-C-183c) (12). These are detailed in the appendix (19.3). Cultured fibroblasts will be transduced during log phase of growth using a retroviral vector containing the gene for human IL-4. Twenty-four hours after transduction, they will be exposed to the selective pressure of 0.3 mg/ml of G418. This concentration may be increased to as much as 1 mg/ml depending on the health of the culture.

3.3 Preparation of the IL-4 Vector-Containing Supernatant:

Supernatants from GTI will be prepared with retroviral vectors incorporating the IL-4 gene (appendix 19.4). They will be tested and shown to be free of mycoplasma and replication-competent virus using the NIH 3T3 and S+/L- assays.

3.4 Preparation and Administration of Vaccine:

When the total population of fibroblasts reaches about 5 X 10^7 cells, the medium will be changed and the amount of IL-4 appearing in the culture supernatant of representative flasks over 24 hours will be determined by ELISA. This will be used to estimate the amount of IL-4 that may be elaborated at each vaccine site per 10^7 cells per 24 hours. About 2 X 10^7 fibroblasts will then be harvested by brief trypsinization for vaccine preparation. At the same time, the frozen tumor cell suspension will be thawed and washed. The number of viable tumor cells will be counted by trypan blue exclusion. Suspensions with less than 50% viability will have viable cells separated with Ficoll-Hypaque centrifugation.

In order to realize the stated objectives of this protocol, patients will first undergo primary vaccination at multiple sites using vaccines made of a fixed number of irradiated tumor cells but escalating numbers of transduced fibroblasts that will deliver varying doses of IL-4 to each vaccine site. This will define the local and systemic toxicity associated with IL-4-elaborating vaccines of different histologies. Five vaccines will be prepared for primary immunization by admixing 5 X 10^6 tumor cells with 3 log dilutions of fibroblasts with the most potent vaccine containing up to 10^7 fibroblasts elaborating no more than 10^5 U of IL-4 per 24 hours. In addition to a nontransduced control vaccine, for example, a patient would receive 4 vaccines that elaborated 10^5, 10^4, 10^3, or 10^2 U IL-4/ 24 hours. Each vaccine will be prepared in 0.1 ml of sterile saline. The vaccine will be irradiated with 5,000 rads prior to intradermal administration. At the time of vaccine administration, a small aliquot of fibroblasts will be set aside to test for lack of growth and for production of IL-4 following irradiation.

Five sites for vaccination, oriented vertically and positioned 2 cm apart, will be placed over the left lumbar region of the patient's back between 2 points tattooed using India ink. This indelible tattoo will allow for the accurate

cells) have shown that 10-20 million cells contain a broad repertoire of immune specificities (D.E. Mosier, personal communication; Bordignon, see letter).

The patients will receive approximately six monthly infusions of autologous peripheral blood lymphocytes which have been transduced with the LASN vector. These infusions will be given within a few days of gene introduction and before the cell population has had the opportunity to proliferate to a significant extent. We anticipate that from 1 to $100x10^6$ cells/kg will be given with each infusion beginning with the cells obtained from 7 ml blood/Kg. This initial cell dose is about 1% the dose of cells given in TIL therapy. A comprehensive immunologic evaluation will be performed at the end of this series of infusions. (Section 8.2) The occurrence of any grade 3 or 4 toxicity associated with the cell infusion that is not easily reversed with medication will be a decision point in the protocol. The iv route of cell infusion will cease being used. The ip route will be instituted at this point.

The child will have recently voided or the position of the bladder will be visualized by ultrasound. The skin of the abdominal midline will be prepared and draped following standard procedures for paracentesis. After administration of local anesthesia, an 18-22 gauge angiocath will be carefully inserted into the peritoneum at or adjacent to the midline below the umbilicus. (71) After the cell infusion is complete, the catheter will be removed and the patient observed closely for at least 1 hour.

Adults receiving cellular immunotherapy with TIL for the treatment of malignant melanoma have been routinely given infusions of $2\text{-}4x10^{11}$ cultured T cells (up to $6x10^9$/kg) and IL2 in a single day. These adults have experienced symptoms ranging from no noticeable effects to various symptoms including chills, fever, lethargy, tachycardia, bradycardia, hypotension, shortness of breath, nausea and vomiting. In most of these cases the symptoms are moderated by premedication and they are seldom severe.

Children with SCID treated by bone marrow transplantation have been given up to 10^9 bone marrow cells/kg intravenously, usually without complications. Even though the initial cell infusions during parts 1 and 2 of this protocol involve small numbers of cells which are unlikely to cause untoward effects, these infusions will be carried out in the Pediatric-ICU to permit more intensive monitoring of the patient's response to the infusion. When sufficient experience has been gained with this protocol, the PI may elect to perform the cell infusions on

localization of the vaccine site for subsequent biopsy in the event a local reaction is not apparent. 0.1 ml of vaccine will be injected intradermally at each marked site using a 1 ml syringe and a 27 gauge needle. After 2 weeks, the 5 sites will be excisionally biopsied and evaluated to determine the dose of IL-4 associated with the most intense mononuclear cell infiltrate.

This procedure will be repeated in 2 weeks provided that the patient has not suffered a severe local reaction such as abscess or ulceration. At this time, 5 vaccines will be placed over the right lumbar region of the patient's back after the sites have been marked as before. Each vaccine will consist of 5×10^6 tumor cells admixed with as many as 10^7 fibroblasts to provide the maximum dose of IL-4 that was found to be free of significant toxicity in the primary vaccination. All sites of secondary vaccination will be sequentially biopsied 1, 2, 4, 7, and 14 days later to determine the kinetics of the immune response induced by the vaccine. This may help to define the proper time to begin systemic IL-2 therapy in subsequent protocols. We anticipate that concomitant IL-2 will be necessary to generate a systemic antitumor response from the local immune response at the vaccine site. If administered too early, IL-2 may attenuate the immune response to the vaccine in view of the previously documented ability of IL-2 to decrease DTH reactions to common recall antigens (19).

3.5 Vaccine Testing:

Prior to administration of the vaccine, the following evaluations will be performed:

A. Quantitation of IL-4 secretion by transfected fibroblasts- At least 10 U/10^7 cells/24 hours will need to be produced. Up to 10^7 cells will be delivered with the vaccine to give a maximum of 10^5 U/24 hours/vaccine site. This upper limit is based on our previous IL-4 protocol in which patients tolerated more than 10^6 U/day given as an IV bolus.

B. Microbiology testing for sterility- After each split of the fibroblasts (approximately once a week), cultures will be sent to the microbiology laboratory to detect possible contamination by bacterial organisms. Mycoplasma testing will be performed one week before infusion by a commercial testing service.

C. S+/L- assay including 3T3 amplification

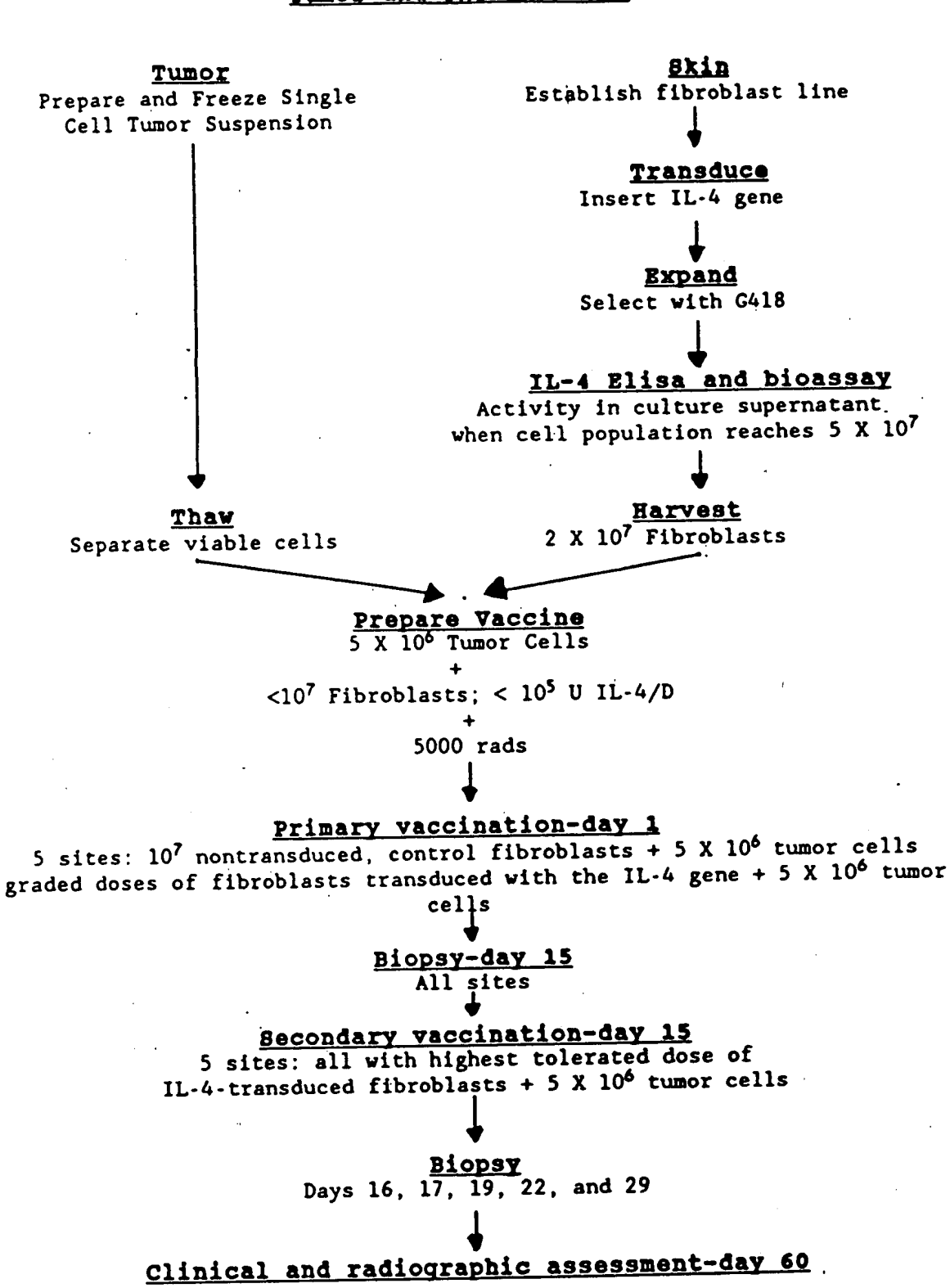
D. Cell viability- Tumor preparation must be at least 50% viable following thawing; if not, Ficoll-hypaque separation to eliminate dead cells may be carried out.

3.6 Immunologic Monitoring:

Fifty ml of blood will be collected in four green top tubes and 1 red top tube on days 0, 7, 14, 21, and 28. Peripheral blood mononuclear cells will be cryopreserved at -180° C and serum will be frozen at -20°C. These specimens will be used to detect alterations in the phenotype of circulating lymphocytes and to quatitate systemic IL-4 levels, respectively. Patients will be evaluated daily

in the clinic for 1 week after each vaccine is administered. The vaccination sites will be assessed for the presense of induration, erythema, tenderness, ulceration, or infection. Patients' temperatures and vital signs will also be monitored. The area of erythema and induration will be measured. Vaccination sites will be excised as described above under local anesthesia in sterile fashion using a 4-6mm punch biopsy knife. The specimens will be divided into thirds. One third will be snap frozen in OCT medium and used for in situ hybridization (appendix 19.5) and immunohistochemistry (appendix 19.6). Another portion (1/3) will be prepared for electron microscopy (appendix 19.7). The remaining third will be placed in tissue culture with 6000 IU/ml IL-2 and complete medium .

4.0 PROTOCOL SCHEMA



the regular patient care unit.

This protocol is classified as research involving greater than minimal risk but presenting the prospect of direct benefit to the individual subject. Discomforts to the patient may include venipuncture and/or other modes of vascular access. Local anesthesia and sedation may be required for the intraperitoneal infusions. The patient may experience chills, fever, tachycardia, nausea and vomiting, and/or shortness of breath during the cell infusion. Potential risks include cardiac arrhythmias, vascular thrombosis, pulmonary embolus, inadvertent infusion of contaminated cultures or mislabeled cells. Intraperitoneal infusions could produce bowel perforation and subsequent serious peritoneal infection. Potential risks with the gene insertion portion of the proposal include the inadvertent contamination of the retrovirus preparation with replication competent murine retrovirus generated by a recombination event occurring in the vector virus packaging cell line. During retroviral-mediated gene transfer the cultured T cells could also undergo an insertional event causing the malignant transformation of the cell (see section 1.5.5). The cultured T cell population could theoretically contain cell sub-populations with potential undesirable consequences for the patient such as autoreactive cells. Cancer patients treated with IL2 infusions alone or with IL2 plus $2\text{-}4x10^{11}$ cultured expanded autologous T cells have experienced transient symptoms which may reflect immune phenomena such as joint aches (not arthritis) and skin rashes. Less than 1/3 of patients have experienced any symptoms and each has been associated with IL2 treatment alone at a similar frequency so that the potential contribution by the T cell infusions is unclear.

The NeoR gene encodes for an enzyme (NPT-II) which inactivates the antibiotics Neomycin and Amikacin. NPT-II does not inactivate other aminoglycoside antibiotics (such as Gentamicin and Tobramycin) and many other suitable alternatives for gram negative infections are available for clinical use. We have observed no untoward effects of expression of the NeoR gene in T cells either *in vitro* or in mice, monkeys, or humans who have received T cells expressing this enzyme.

Adverse drug reactions (ADRs) to the IND Drug will be reported promptly to the Investigational Drug Branch (IDB), phone (301) 496-7957. ADR reports are required even if there is only a suspicion of a drug effect. Previously unknown Grade 1,2 and 3 reactions will be reported to the NCI in writing using the "NCI Adverse Reactions Form for Investigational Agents" within 10

working days. Grade 4 (life-threatening) reactions and patient deaths while on treatment will be reported to the NCI by phone within 24 hours. A written report will follow within 10 working days.

Written reports will be sent to:

Investigational Drug Branch
Cancer Therapy Evaluation Program
P.O.Box 30012
Bethesda, MD 20824

The end-point for part 1 will occur when the patient has received approximately 6 infusions of transduced, non-selected cells. A thorough immunologic evaluation will be performed at this time. All patients will progress to part 2.A. of the protocol. Part 1 will require approximately 6 patient admissions to the Clinical Center and will last from 6-9 months. Part 1 may result in significant benefit to the patient from a measurable improvement in immune function or clinical status. We will also be able to evaluate the survival in the patient's blood of the infused ADA gene-modified T cells and to determine whether autologous T cell infusions may have a selective growth advantage *in vivo.*

7.2 Part 2: Infusion of Transduced Cells Selected for Expression of ADA.

Part 2.A. of this study will involve approximately six monthly infusions of transduced lymphocytes selected for expression of the introduced genes. The procedure and possible complications of this part are identical to those in part 1. Again, cells will be infused as quickly as selection provides a transduced lymphocyte population with a normal blood level of ADA. Serial sampling of the patient's blood to insert the ADA gene should permit genetic correction of a broad selection of the patient's T cell repertoire since presumably different immune specificities will be present in different proportions at the different time points.

In part 2.B., the dose of infused gene-transduced selected lymphocytes will be escalated at approximately monthly intervals in half log increments from 10^8/kg to reach a target level of 1-3 $x10^9$/kg. The patients will then receive approximately 6 monthly infusions of selected cells at this cell concentration.

5.0 PATIENT SELECTION

5.1 Eligibility:

A. Patients must have histologically confirmed metastatic, and/or unresectable, locally advanced melanoma, renal cell carcinoma, breast cancer or colon cancer for which curative measures do not exist or are no longer effective. Patients must have expected survivals of 6 months or less. Patients must be off all therapy for at least 1 month prior to beginning treatment.

B. Women of child-bearing potential must have a negative pregnancy test.

C. Patients must have a negative HIV test.

D. Patients must have intact cell mediated immunity as manifest by a positive response to any one of a broad panel of skin tests including TB, mumps, candida, and SKSD (Merieux skin test). Skin tests will be read at 24 and 48 hours.

E. Patients must be free of systemic infection.

F. Patients must have the following laboratory values:

1. WBC > $2000/mm^3$

2. Platelet count > 50,000

3. Bilirubin < 2mg/dl

4. Creatinine < 2mg/dl

G. Patients must have biopsy proven metastatic melanoma, breast cancer, renal cell cancer, or colon cancer that is measurable by physical exam, or radiologic immaging.

H. Patients must be at least 18 years old.

5.2 Ineligibility:

A. Patients who fail to meet the above criteria.

B. Patients who are hepatitis B surface antigen positive or HIV antibody positive.

C. Patients who require steroids in any form- topical or systemic.

D. Patients with a history of having had another cancer with the exception of basal cell or squamous cell carcinoma of the skin.

E. Patients who have had therapy within 28 days

F. Patients who are pregnant

G. Patients with systemic infections or coagulation disorders

H. Patients with an ECOG performance status >2.

6.0 LOCATION

All patients will be treated on the PCI inpatient or outpatient units of Montefiore University Hospital. All resected tissues, serum, and blood specimens will be processed by the IMDL/CT of the PCI located on the 10th floor of the BST at the University of Pittsburgh.

7.0 PATIENT EVALUATION

7.1 Before Therapy:

A. Complete physical exam including detailed measurement of all metastatic lesions.

B. Blood tests (85 ml) including:

1. electrolytes, BUN, creatinine, AST, ALT, LDH, total bilirubin, direct bilirubin, alkaline phosphatase, glucose, calcium, magnesium, phosphorus, albumin, total protein, CEA (for patients with colon and breast cancer.
2. CBC with differential and platelet counts.
3. PT, PTT.
4. Pregnancy test for women who may conceive.
5. Hepatitis screen
6. HIV screen
7. 10 ml of clotted blood for serum storage
8. 45 ml of blood for mononuclear cell preservation.

C. Urinalysis

D. Chest X-ray

E. EKG

F. Baseline CT scan or MRI of brain, chest, abdomen, and pelvis to evaluate metastatic disease

7.2 After Therapy:

A. Physical examination 1, 2, 4, and 7 days after each vaccination that will include an assessment of the vaccination sites.

B. Weekly blood tests (x 4) including:

1. CBC with differential and platelet counts.

2. Chemistries (see above)

3. PT, PTT

4. Serum (10ml) and PBMC (30ml) for storage

C. Biopsy of vaccination sites on days 14, 15, 16, 18, and 21.

8.0 CRITERIA FOR RESPONSE

Patients will be assessed at one and two months following their second vaccination for evidence of tumor response using physical examination or radiographic studies. Responses will be defined as follows:

A. Complete response- disappearance of all measurable disease for at least 1 month without the development of new lesions.

B. Partial response- 50% or greater decrease in the sum of the products of the perpendicular diameters of all measurable lesions lasting at least 4 weeks with no increase in the size of existing lesions or appearance of new lesions.

C. No response- any patient not manifesting a complete or partial response.

9.0 REPORTING OF ADVERSE REACTIONS

All side effects will be graded using the standard toxicity sheet used in prior immunotherapy protocols presented in appendix 18.7. Adverse reactions will be reported promptly to the Investigational Drug Branch at (301) 496-7957. Reports are required even if there is only a suspicion of an adverse effect. Previously unknown grade 2 and grade 3 reactions will be reported to the NCI in writing using the "NCI Adverse Reactions Form for Investigational Agents" within 10 working days. Grade 4 (life-threatening) reactions and patient deaths while on treatment will be reported to NCI by phone within 24 hours. A written report will follow within 10 working days. Written reports will be sent to:

Investigational Drug Branch
Cancer Therapy Evaluation Program
P.O. Box 30012
Bethesda, Maryland 20824

All adverse reactions should also be reported to the IRB. Data will be submitted to CTMS at least once every two weeks. The NCI/DCT case report or ACAS will be used to report to CTMS.

10.0 POTENTIAL RISKS OF RETROVIRAL-MEDIATED GENE MODIFICATION

10.1 Insertional Mutagenesis:

The possibility of causing malignancy in cells secondary to the random insertion of the retroviral vectors in the genome exists, though the actual risk of this occurring is thought to be low. Nevertheless, transduced fibroblasts will be irradiated with 5,000 rads prior to vaccination. Tests of the viral supernatant as well as of the fibroblasts used for vaccination will be conducted to assure that no replication competent virus is present in either preparation. Since no other cells will be exposed to retroviral vector insertion, no other cells will be at risk for insertional mutagenesis.

10.2 Risk from Murine Retrovirus:

Exposure of the cancer patient to retrovirus could theoretically pose a risk of insertional mutagenesis. It should be emphasized, however, that careful tests will be conducted to assure that the patient is not exposed to replication competent virus. The retrovirus derived from the Moloney murine leukemia virus has been modified so that it no longer contains any intact viral genes and thus cannot produce the proteins necessary to package its RNA into an intact infectious virus (14, 15). To assemble the retrovirus, a retrovirus packaging cell line will be used that contains coding sequences that express the viral structural proteins. This packaging cell line does not produce replication competent retrovirus because of multiple modifications made to the gag, pol, and env coding sequences that prevent its replication. These modifications include removal of signals required for RNA encapsidation, priming of reverse transcription, and integration. Multiple assays will be performed on the packaging cell line, the retroviral vector supernatant as well as on the fibroblasts prior to vaccination to insure that no replication competent virus is present. These tests will include S+/L- assays and 3T3 amplification. Any supernatants or fibroblasts with evidence of any replication competent virus will not be utilized. The 3T3 amplification and S+/L- assays are thought to be capable of detecting a single replication competent viral particle per ml. These studies will initially be done at GTI and then subsequently in the IMDL/CT.

Prior safety studies have shown that exposure of primates to large infusions of infectious murine amphotrophic virus produce no acute pathologic effects (16). In a study of 21 primates receiving retroviral-mediated, gene-modified, autologous bone marrow cells no animal showed evidence of toxicity related to the gene transfer for as long as 4 years after infusion (17).

Patients in the proposed protocol will not be exposed to the vector supernatant. Fibroblasts will be transduced with the retroviral vector supernatant and then washed extensively. They will be grown for several weeks in the absence of supernatant. The fibroblasts will then be washed extensively again prior to reinfusion into the patient.

11.0 STATISTICAL CONSIDERATIONS

11.1 Analysis of Peripheral Blood Specimens

Systemic IL-4 levels and the phenotype of circulating peripheral blood lymphocytes will be determined on days 0 (pre-vaccination), 7, 14, 21, and 28. Follow-up multiple comparisons, based on either the paired-t or signed rank statistic, will be applied in the interpretation of significant results.

11.2 Analysis of Local Toxicity

The incidence of severe, vaccine-related, local reactions will be recorded. The relationship between local toxicity and the number of transferred cells and the amount of IL-4 elaborated per site per day will be determined. A formal test for increasing dose-response may be based on a scored-rank test with p-values obtained by within-patient permutation of dose levels. To insure the validity of this approach, and more importantly to eliminate the possibility of positional bias, the relative location of vaccinations will be randomized in double-blind fashion for each patient.

11.3 Dose-Response for Mononuclear Cell Infiltration

Biopsies obtained after primary vaccination on day 14 will provide an opportunity to quantify the relationship between the amount of IL-4 delivered and the degree of infiltration by mononuclear cells. These data will first be investigated by standard repeated measures methods; but, because the description of between-patient variability both in level of response and in optimal dose are of considerable interest, additional analyses are planned as described below.

It is thought likely that the dose-response relationship may not be monotonically increasing, but rather may re-descend at sufficiently high levels of IL-4. The distribution over patients of the maximal response and of the maximizing dose will be tabulated. These quantities will be estimated for each patient under an assumption of unimodal dose-response curves. Briefly, a pooled-adjacent-violators algorithm will be used, but adapted to unimodal rather than monotonic curves. A formal test of dose-response may be based on a permutation test whose statistic is an aggregation of the deviations between each patient's estimated maximum and his or her average level of infiltration. Comparison of IL-4 vaccinations against the nontransduced control may be based on a similar strategy.

A similar approach may be used to analyze the dose-response relationship for other endpoints, including local endothelial activation and cytokine production.

11.4 Kinetics of T-Cell Infiltration

Serial biopsies of secondary vaccination sites on days 15, 16, 18, 21, and 28 will help to define the time course of the local immune response. These data will be analyzed in a manner similar to that described in 11.3.

11.5 Clinical Response

The limited number of patients to be accrued (20) together with their varied histologies (4) will limit the generalizability of the response rates that will be observed. Rates of response will be tabulated and compared to relevant norms, but no formal statistical analysis will be carried out.

12.0 ETHICAL CONSIDERATIONS

Dr. Rosa Lynn Pinkus (Medical Ethics) will assist in developing and reviewing all protocol consent documents with the principal investigators. Every attempt will be made to protect the patients' and their family's privacy. Informed consent in this project is considered to be a process of shared decision-making between patient and physician. All written consents will be discussed with this concept in mind.

13.0 RESPONSIBILITIES

13.1 Consent- Drs. Lotze or Rubin.

13.2 Tumor harvesting from lung- Drs. Ferson or Landreneau

13.3 Tumor harvesting from subcutaneous sites- Drs. Lotze, Rubin, Posner, or Edington

13.4 Biopsy of vaccination sites- Drs. Lotze, Rubin, Posner, or Edington

13.5 Preparation of tumor cell suspensions, culturing of fibroblasts, retroviral gene transduction, and preparation of vaccine- Immunologic Monitoring and Cellular Therapy Laboratories

13.6 Administration of vaccine- Drs. Lotze, Rubin, Posner, or Edington

13.7 Preparation of viral supernatants and safety testing- GTI/PCI

14.0 RISKS AND BENEFITS

Systemic interleukin-4 may cause flu-like symptoms such as fever, chills, fatigue, loss of appetite, weight loss, headache, nasal congestion, nausea, vomiting, gastritis or gastric ulcer, and diarrhea. IL-4 may cause a lowering of the white blood cell count that could increase the risk of infection; it may cause abnormal elevation of liver enzymes, abnormal kidney function and low blood pressure. It is unlikely that any of these toxicities will be induced by the low levels of IL-4 elaborated locally by the vaccine.

Individual patients may benefit by having their tumors shrink or disappear. Symptoms related to cancer may improve if shrinkage of tumor is achieved. The population of patients with cancer may benefit from this trial which may define the role tumor vaccines using cells genetically modified to produce cytokines

such as IL-4.

If there are serious systemic effects of the IL-4 vaccine, which we believe are unlikely to occur, the vaccination site can be surgically excised. As an additional safety factor, fibroblasts have been shown to stop producing transfected gene products in murine models after two weeks.

15.0 COST AND PAYMENTS

Patients and/or their insurance carriers will be expected to pay for the cost of treatment and evaluation. Every effort will be made to ensure that payment will be forthcoming prior to enrolling patients.

16.0 TREATMENT SCHEMA

DAY	1	2	3	4	5	6	7	8//15	16	17	18	19	20	21	22//29
VACCINATE	X							X							
BIOPSY								X	X	X		X		X	X
BLOOD	X							X	X					X	X
CXR	X								X						X
PHYS EXAM	X	X	X		X			X	X	X	X		X		X	X

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